Bastian Daubert Presentation Mock-Up

VIAGRA DAUBERT HEARING

Boris Bastian, M.D., Ph.D.

University of California at San Francisco

Prepared: October 3, 2019

University of California, San Francisco

CURRICULUM VITAE

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Dermatology
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EDUCATION

1981 - 1982	University of Cologne	NA	Chemistry	
1982 - 1989	Ludwig-Maximilian-University of Munich	MD	Medicine	
1985 - 1989	Munich, Medical Sciences Ludwig-Maximilian-University of Munich	Dr. Med	Magna cum laude	Prof. Dr. med. Guder
1987 - 1987	Krankenhaus Harlachingen, Munich, Germany	Intern	Surgery	
1987 - 1987	Dermatologische Klinik, LMU, Munich, Germany	Intern	Dermatology	
1987 - 1988	Schwabinger Krankenhaus,Munich, Germany	Intern	Medicine	
1988 - 1989	Ludwig-Maximilian-University	Fellow	Hematology	
1989 - 1994	University of Würzburg	Resident	Dermatology	
1995 - 1996	University of California, San Francisco	Visiting Scholar	Dermatopathology	
1997 - 1999	University of California, San Francisco; Comprehensive Cancer Center	Fellow	Cancer Genetics Program	

LICENSES, CERTIFICATION

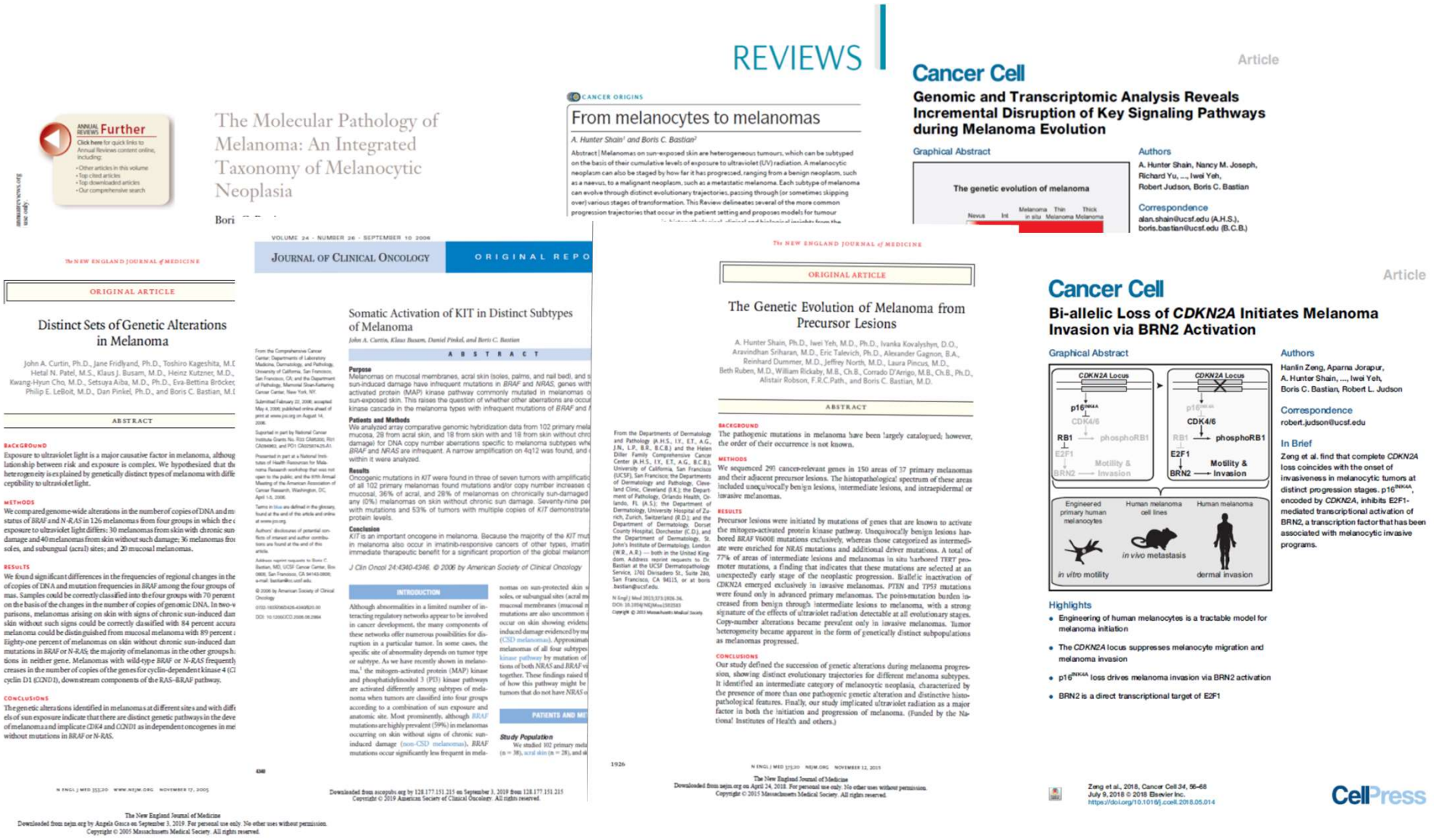
1989 Medical License: Bavarian Medical Board

1 of 65

- Professor of Dermatology and Pathology
- Distinguished Professor of Cancer Biology, Helen Diller Family Comprehensive Cancer Center
- Principal Investigator, Bastian Research Laboratory
- Leader, Cutaneous Oncology Research and Clinical Program
- Director, Molecular Dermatopathology Clinical Service
- Founder and Executive Director, Clinical Cancer Genomics Lab

Boris Bastian, M.D., Ph.D.

Selected Publications on Melanoma Initiation and Progression



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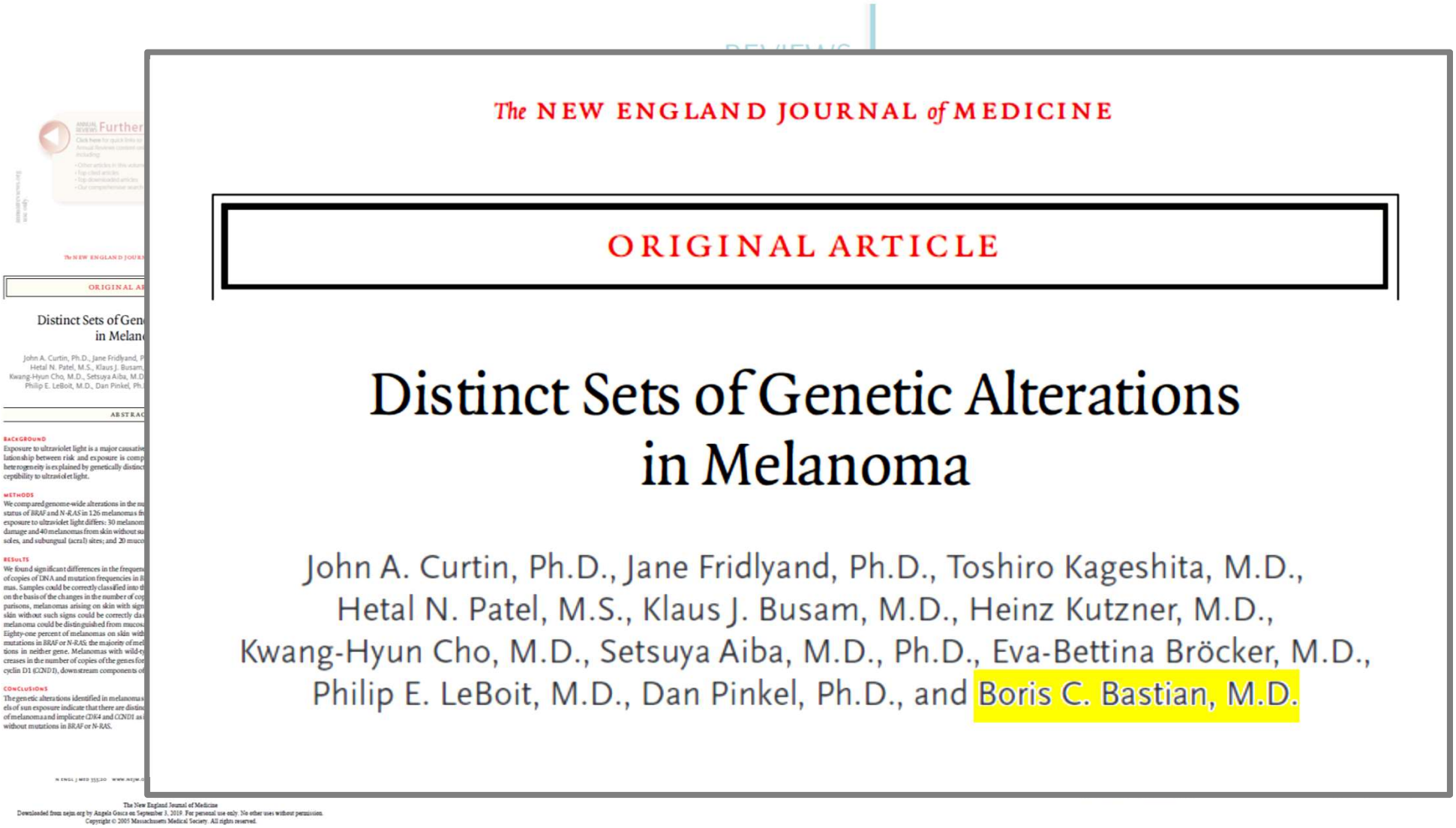


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Distinct Sets of Genetic Alterations in Melanoma

John A. Curtin, Ph.D., Jane Fridlyand, Ph.D., Toshiro Kageshita, M.D., Hetal N. Patel, M.S., Klaus J. Busam, M.D., Heinz Kutzner, M.D., Kwang-Hyun Cho, M.D., Setsuya Aiba, M.D., Ph.D., Eva-Bettina Bröcker, M.D., Philip E. LeBoit, M.D., Dan Pinkel, Ph.D., and Boris C. Bastian, M.D.

Boris Bastian, M.D., Ph.D.

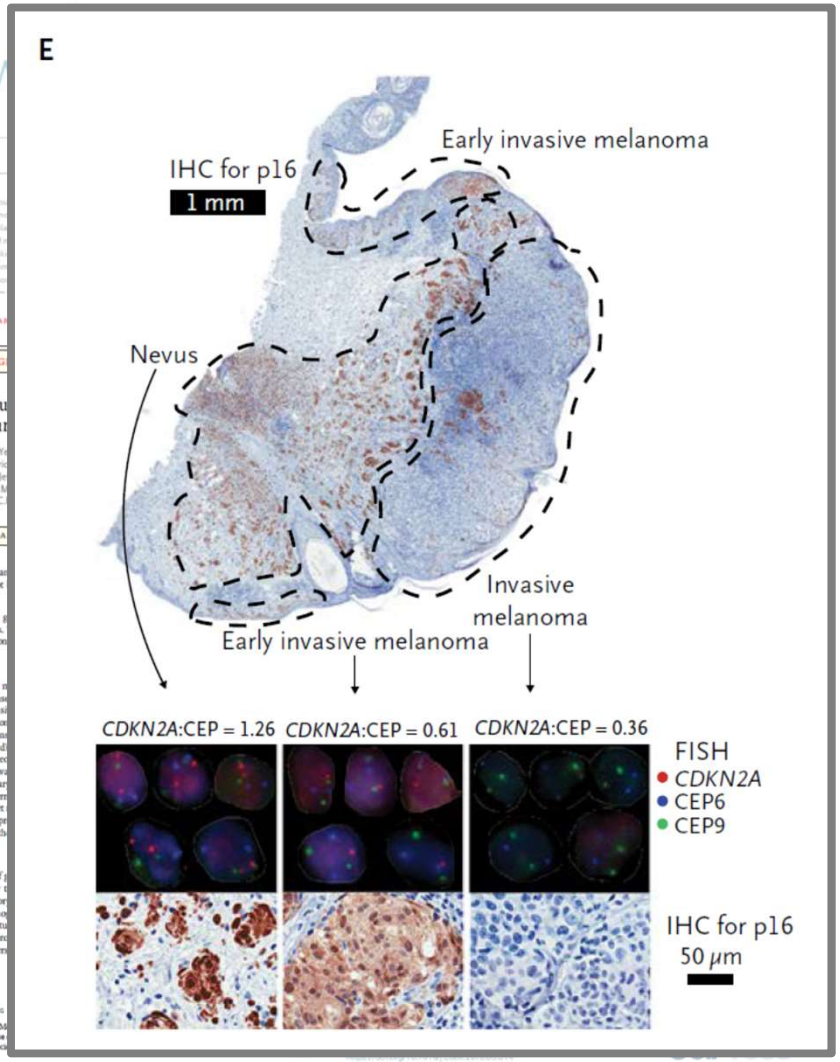
Selected Publications on Melanoma Initiation and Progression

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

The Genetic Evolution of Melanoma from Precursor Lesions

A. Hunter Shain, Ph.D., Iwei Yeh, M.D., Ph.D., Ivanka Kovalyshyn, D.O., Aravindhan Sriharan, M.D., Eric Talevich, Ph.D., Alexander Gagnon, B.A., Reinhard Dummer, M.D., Jeffrey North, M.D., Laura Pincus, M.D., Beth Ruben, M.D., William Rickaby, M.B., Ch.B., Corrado D'Arrigo, M.B., Ch.B., Ph.D., Alistair Robson, F.R.C.Path., and Boris C. Bastian, M.D.



Boris Bastian, M.D., Ph.D.

Selected Publications on Melanoma Initiation and Progression

The Molecular Pathology of Melanoma: An Integrated Taxonomy of Melanocytic Neoplasia

Boris C. Bastian

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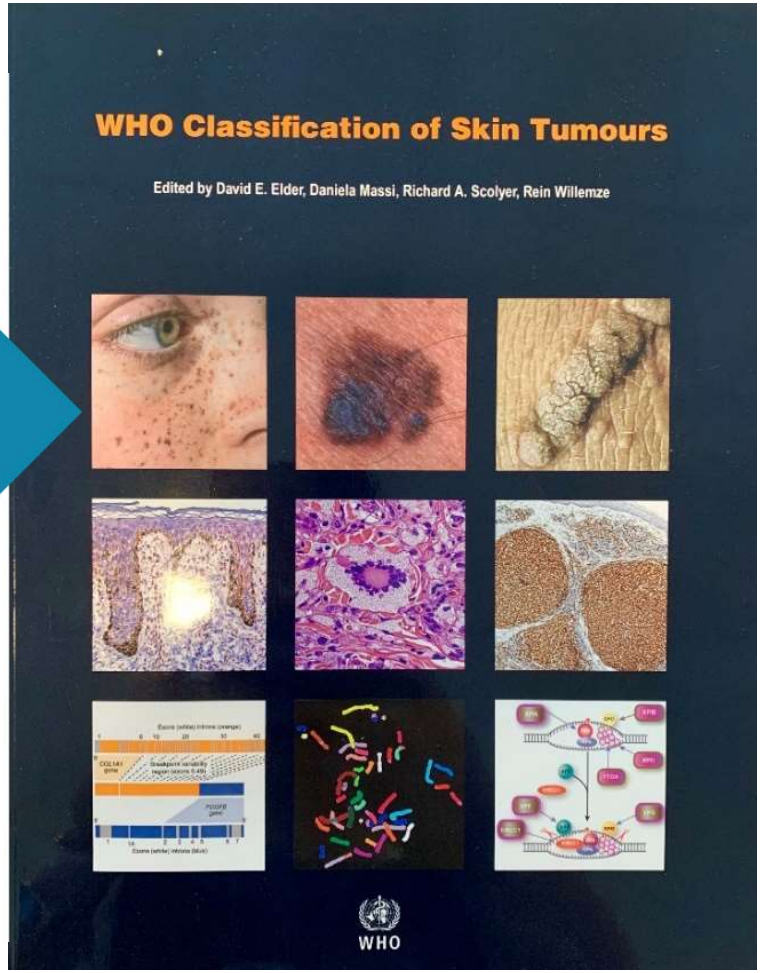
Annu. Rev. Pathol. Mech. Dis. 2014. 9:239–71
The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org
This article's doi:
10.1146/annurev-pathol-012513-104658

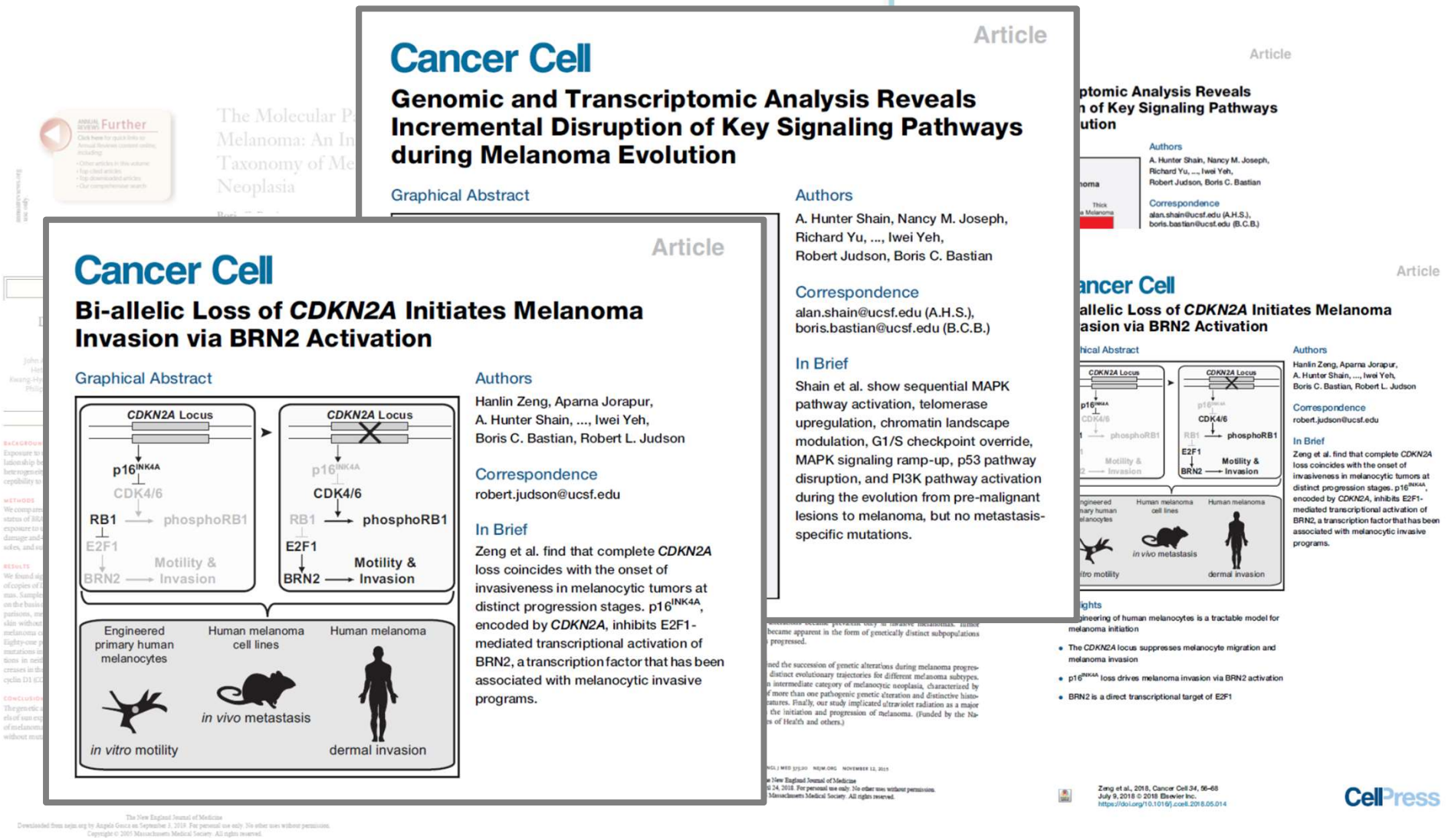
Keywords
genetics, pathogenesis, classification, mutation, nevi

Abstract
Melanomas comprise multiple biologically distinct categories, which differ in cell of origin, age of onset, clinical and histologic presentation, pattern of metastasis, ethnic distribution, causative role of UV radiation, predisposing germ-line alterations, mutational processes, and patterns of somatic mutations. Neoplasms are initiated by gain-of-function mutations in one of several primary oncogenes, which typically lead to benign melanocytic nevi with characteristic histologic features. The progression of nevi is restrained by multiple tumor-suppressive mechanisms. Secondary genetic alterations override these barriers and promote intermediate or overtly malignant tumors along distinct progression trajectories. The current knowledge about the pathogenesis and clinical, histologic, and genetic features of primary melanocytic neoplasms is reviewed and integrated into a taxonomic framework.

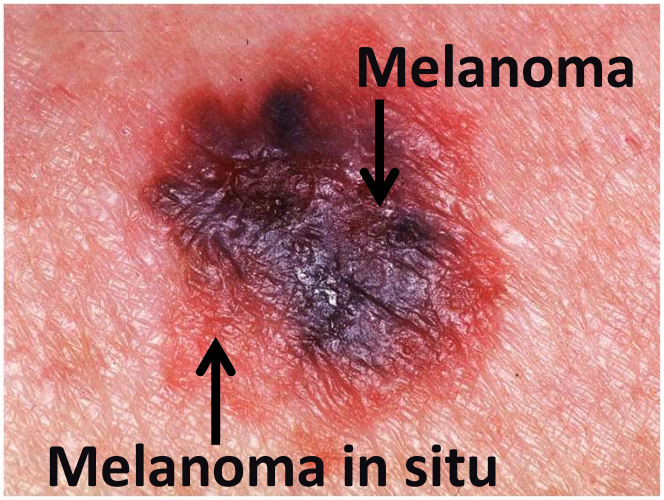


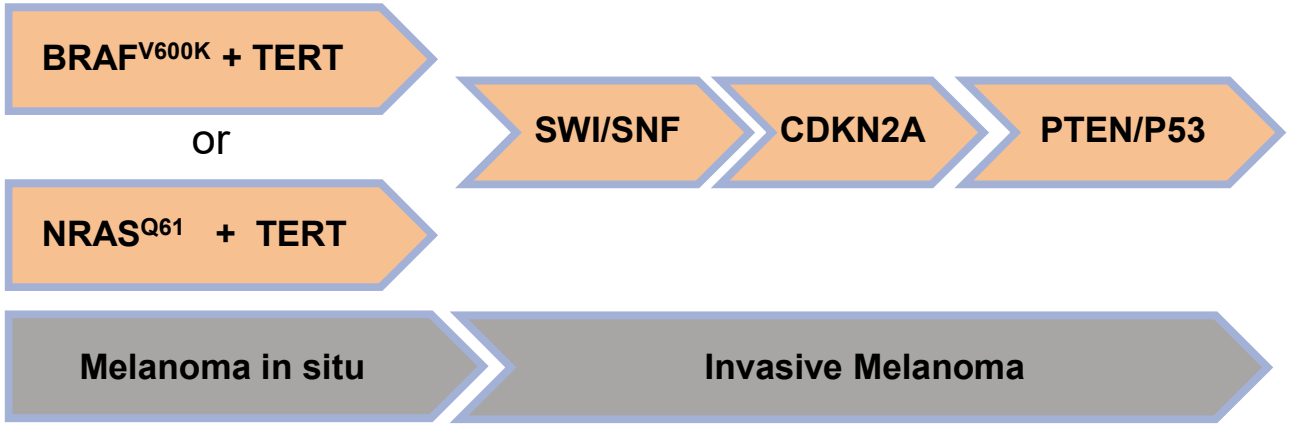
Boris Bastian, M.D., Ph.D.

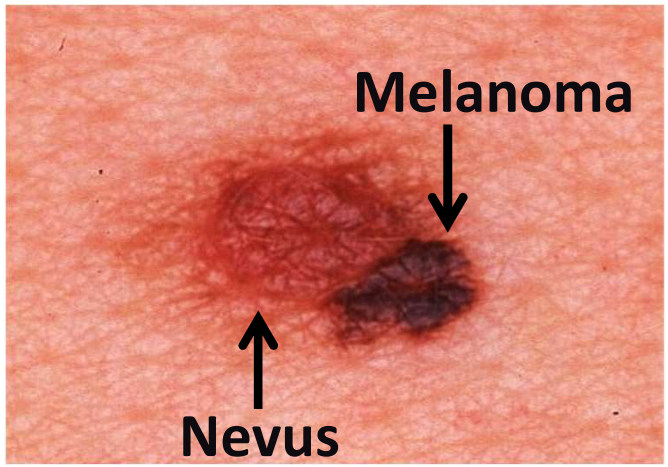
Selected Publications on Melanoma Initiation and Progression

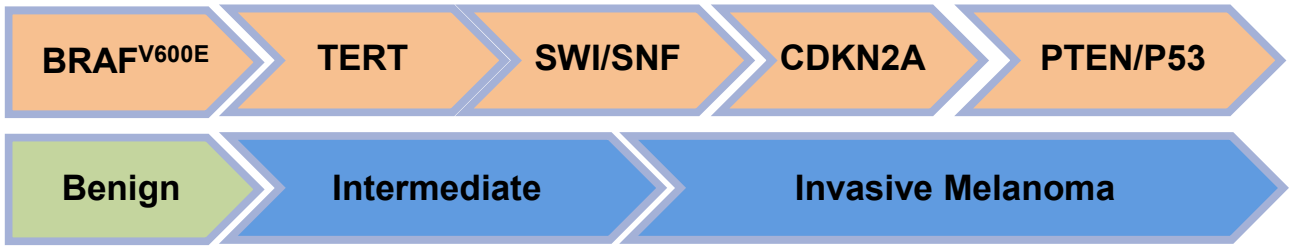


A Stepwise Accumulation of Mutations Causes Melanoma Initiation and Progression

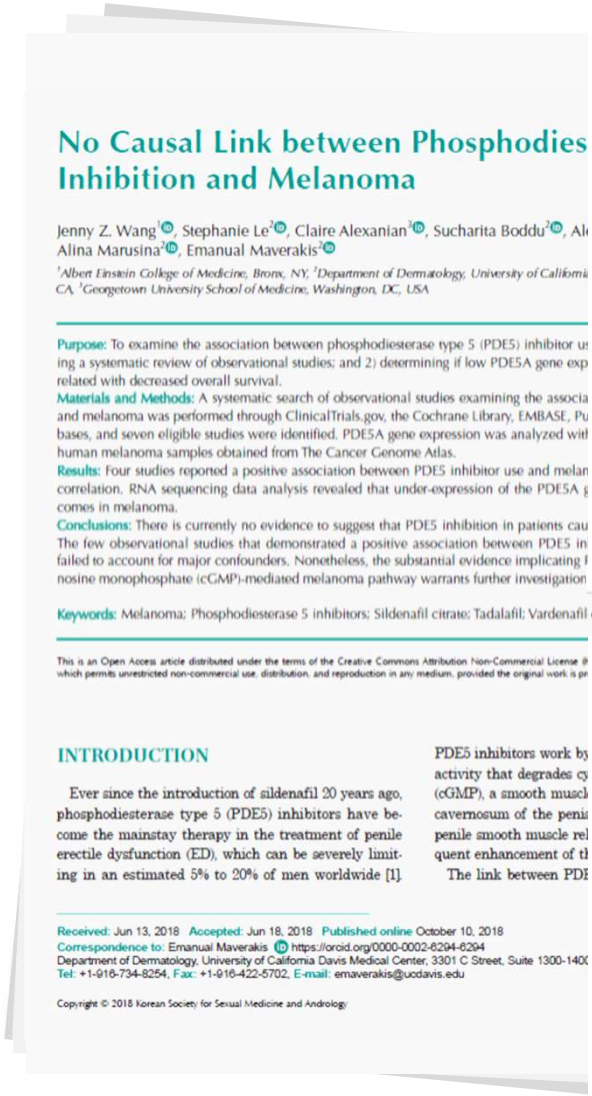








"No Causal Link between Phosphodiesterase Type 5 Inhibition and Melanoma"



No Causal Link between Phosphodies
Inhibition and Melanoma

Jenny Z. Wang[1], Stephanie Le[2], Claire Alexanian[3], Sucharita Boddu[2], Al
Alina Marusina[2], Emanual Maverakis[2]

[1]Albert Einstein College of Medicine, Bronx, NY, [2]Department of Dermatology, University of California
CA, [3]Georgetown University School of Medicine, Washington, DC, USA

Purpose: To examine the association between phosphodiesterase type 5 (PDE5) inhibitor us
ing a systematic review of observational studies; and 2) determining if low PDE5A gene exp
related with decreased overall survival.
Materials and Methods: A systematic search of observational studies examining the associa
and melanoma was performed through ClinicalTrials.gov, the Cochrane Library, EMBASE, Pu
bases, and seven eligible studies were identified. PDE5A gene expression was analyzed with
human melanoma samples obtained from The Cancer Genome Atlas.
Results: Four studies reported a positive association between PDE5 inhibitor use and melan
correlation. RNA sequencing data analysis revealed that under-expression of the PDE5A g
comes in melanoma.
Conclusions: There is currently no evidence to suggest that PDE5 inhibition in patients cau
The few observational studies that demonstrated a positive association between PDE5 in
failed to account for major confounders. Nonetheless, the substantial evidence implicating
nosine monophosphate (cGMP)-mediated melanoma pathway warrants further investigation

Keywords: Melanoma; Phosphodiesterase 5 inhibitors; Sildenafil citrate; Tadalafil; Vardenafil dihydrochloride

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INTRODUCTION

Ever since the introduction of sildenafil 20 years ago, phosphodiesterase type 5 (PDE5) inhibitors have become the mainstay therapy in the treatment of penile erectile dysfunction (ED), which can be severely limiting in an estimated 5% to 20% of men worldwide [1].

PDE5 inhibitors work by
activity that degrades cy
(cGMP), a smooth muscl
cavernosum of the penis
penile smooth muscle rel
quent enhancement of th
The link between PDE

Received: Jun 13, 2018 Accepted: Jun 18, 2018 Published online October 10, 2018
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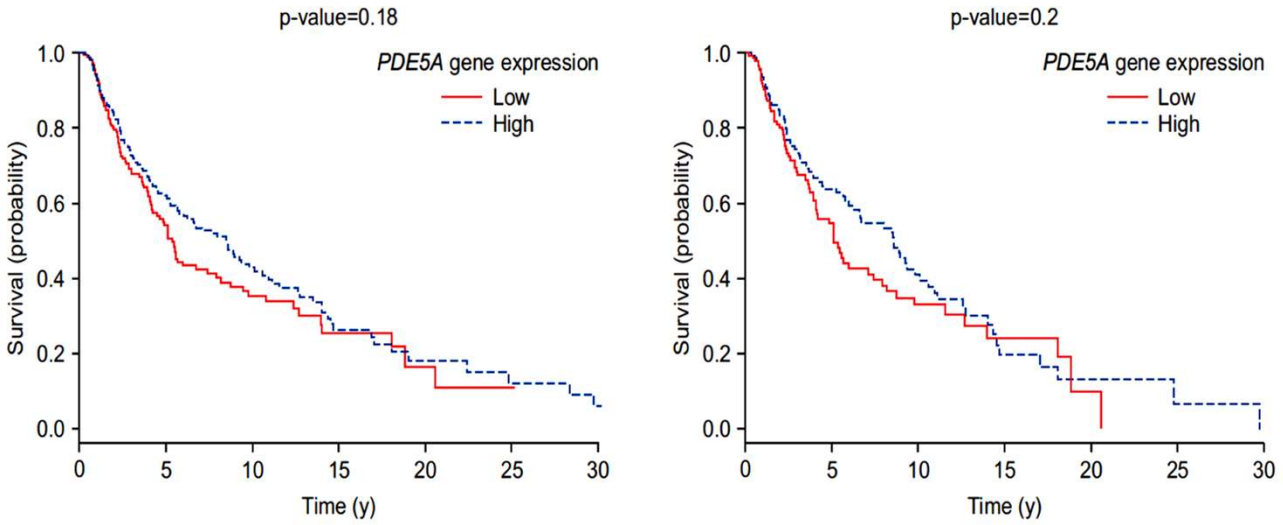


Fig. 1. Kaplan-Meier survival curve for differential expression levels of PDE5A gene in (A) **470 patients** (180 females and 290 males) with a diagnosis of melanoma at any tumor stage (0–IV), ages 14–91 years; and (B) specifically male melanoma patients (n=287) with a diagnosis of melanoma at any tumor stage (0–IV), ages 18–91 years. **Melanoma prognosis unaffected by high or low PDE5A expression in patients, regardless of gender, age, or tumor stage**.

Arozarena *In Vivo*: No Invasion Effect

Cancer Cell
Article

Oncogenic BRAF Induces Melanoma Cell Invasion by Downregulating the cGMP-Specific Phosphodiesterase PDE5A

Imanol Arozarena,[1,3] Berta Sanchez-Laorden,[1] Leisl Packer,[1] Cristin Amaya Viros,[1] Erik Sahai,[2] and Richard Marais[1,*]
[1]The Institute of Cancer Research, Signal Transduction Team, Section o
[2]Tumour Cell Biology Laboratory, CR-UK LRI, 44 Lincoln's Inn Fields,
[3]Present address: Faculty of Life Sciences, Michael Smith Building, Ur
*Correspondence: richard.marais@icr.ac.uk
DOI 10.1016/j.ccr.2010.10.029

SUMMARY

We show that in melanoma cells oncogenic BRAF, act
downregulates the cGMP-specific phosphodiesterase
small decrease in proliferation, its major impact is to sti
This is because PDE5A downregulation leads to an incr
Ca^{2+}, stimulating increased contractility and inducing i
increase in short-term and long-term colonization of t
pathway in NRAS mutant melanoma or BRAF mutant c
oncogenic BRAF induces invasion through downregul

INTRODUCTION

Melanocytes are specialized pigment cells located primarily in the skin, where they determine complexion and hair color and provide protection from the damaging effects of ultraviolet radiation (Gray-Schopfer et al., 2007; Kasper et al., 2007). These cells are also the precursors of melanoma, a potentially deadly skin cancer that kills about 8,000 people in the United States and about 12,000 people in Europe each year. In many Western societies, melanoma incidence almost doubles every decade. If treated early, melanoma can be cured by surgical resection, but due to its proclivity to metastasize, in about 20% of patients it progresses to an aggressive invasive disease that is refractory to treatment and has a poor prognosis, with median survival rates of 6–9 months and 5 year survival rates of 5%–10%. These data highlight the need for improved understanding of melanoma biology to facilitate development of therapeutic strategies.

An important signaling pathway in melanoma is the RAS/RAF/MEK/ERK cascade (Gray-Schopfer et al., 2007). RAS is a small G

Significance

The protein kinase BRAF is activated by somatic gain-of-fu
results in ERK pathway hyper-activation, and we show that thi
phodiesterase. PDE5A is the target of drugs such as sildena
dysfunction and pulmonary arterial hypertension. PDE5A do
in vitro and in vivo and increased long-term colonization of t
induces invasion in vitro but does not increase lung coloniz
and Ca^{2+} metabolism, and the regulation of invasion by oncog

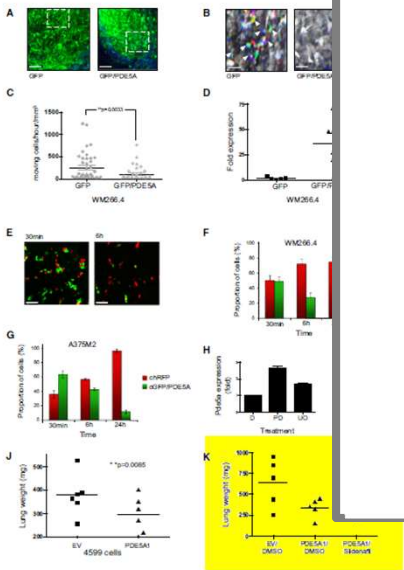
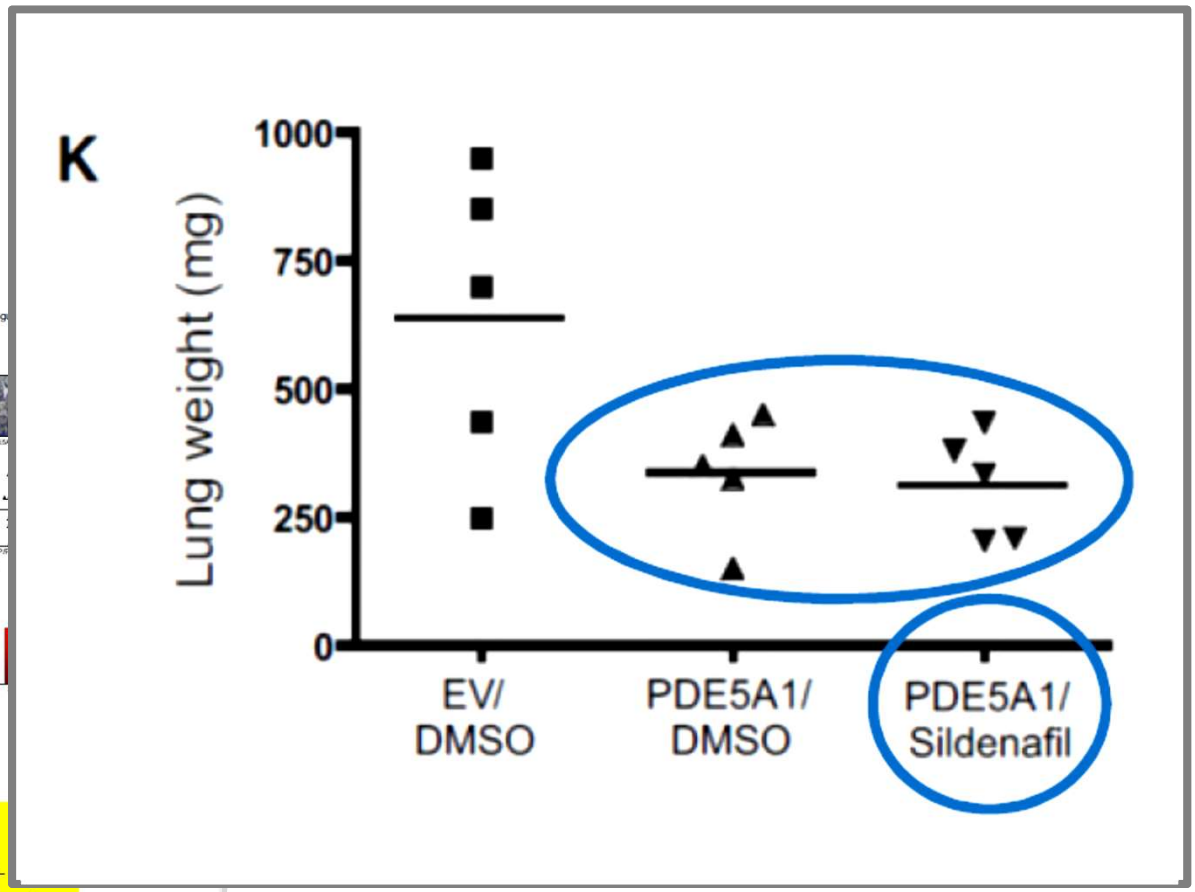
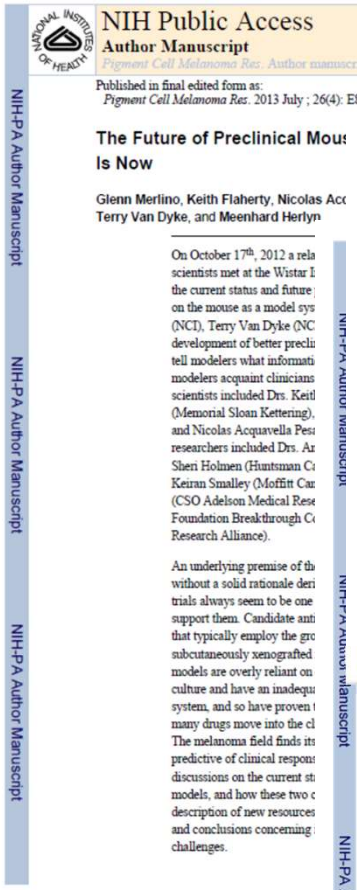


Figure 7. PDE5A Regulates Melanoma Cell Invasion In Vivo

(A) Low-resolution still images taken from video recordings of subcutaneous tumors formed from WM266.4-GFP (GFP) or WM266.4-GFP/PDE5A (GFP/PDE5A) cells. Scale bar, 80 μm. (B) High-resolution images of the region highlighted in the dotted boxes in (A). Three images of the cells were taken at 0, 11, and 22 min, false colored red, green, and blue, respectively, and then overlaid. Scale bar, 30 μm. (C) Quantification of moving cells (cells/hr/mm²) of 40 movies from 8 tumors formed using WM266.4-GFP (GFP) or WM266.4-GFP/PDE5A (GFP/PDE5A) cells. The solid bars represent the average number of moving cells for the two populations with error bars to represent standard deviations from the mean. (D) PDE5A1 mRNA expression in WM266.4-GFP (GFP) or WM266.4-GFP/PDE5A (GFP/PDE5A) tumors was determined by qRT-PCR. Five tumors for each cell type were analyzed in triplicate, and average values for individual tumors are shown, relative to the value for endogenous PDE5A in WM266.4-GFP cells. The bars represent the average level for each tumor group. (E) Fluorescent images of WM266.4-chRFP (red) or WM266.4-GFP/PDE5A (green) cells in the lungs of mice 30 min or 6 hr after injection with equal number of each line. Scale bar, 75 μm. (F) Quantification of 10 fields of cells/lung from 3 mice 30 min, 6 hr, or 24 hr after injection with equal numbers of WM266.4-chRFP (chRFP) or WM266.4-GFP/PDE5A (GFP/PDE5A) cells. (G) Quantification of 10 fields of cells/lung from 3 mice 30 min, 6 hr, or 24 hr after injection with equal numbers of A375M2-chRFP (chRFP) or A375M2-GFP/PDE5A (GFP/PDE5A) cells. (H) Expression of Pde5a mRNA quantified by qRT-PCR in ^{V600E}Braf-expressing 4599-mouse melanoma cells treated with DMSO (D), PD184352 (PD; 1 μM), or U0126 (U0; 10 μM) for 24 hr. (I) Western blot showing PDE5A1 and ERK2 (loading control) levels in 4599 melanoma cells engineered for stable expression of PDE5A1 or an empty vector (EV) control. (J) Lung weights from mice following tail vein injection of 4599 melanoma cells expressing empty vector (EV) or PDE5A1. The weights of the individual lungs are shown, with the bars representing the mean



Research Community on B16 Mouse Melanoma



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Pigment Cell Melanoma Res. Author manuscript; available in PMC 2014 July 24.

Published in final edited form as:
Pigment Cell Melanoma Res. 2013 July ; 26(4): E8–E14. doi:10.1111/pcmr.12099.

The Future of Preclinical Mous
Is Now

Glenn Merlino, Keith Flaherty, Nicolas Acc
Terry Van Dyke, and Meenhard Herlyn

On October 17th, 2012 a rela
scientists met at the Wistar I
the current status and future
on the mouse as a model sys
(NCI), Terry Van Dyke (NC
development of better preclii
tell modelers what informati
modelers acquaint clinicians
scientists included Drs. Keitl
(Memorial Sloan Kettering),
and Nicolas Acquavella Pes
researchers included Drs. An
Sheri Holmen (Huntsman Ca
Keiran Smalley (Moffitt Can
(CSO Adelson Medical Rese
Foundation Breakthrough C
Research Alliance).

An underlying premise of th
without a solid rationale deri
trials always seem to be one
support them. Candidate anti
that typically employ the gro
subcutaneously xenografted
models are overly reliant on
culture and have an inadequ
system, and so have proven t
many drugs move into the cl
The melanoma field finds its
predictive of clinical respons
discussions on the current st
models, and how these two c
description of new resources
and conclusions concerning
challenges.

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However, current in depth knowledge about the complex molecular basis of human melanoma has revealed that this well-established and widely used cell line [B16] **does not reflect the genetic underpinnings of human melanoma**.

the cognate immune system for any model may be paramount in developing accurate
response hypotheses.

For more than five decades, the tumor immunology field has greatly benefited from the development of the poorly immunogenic B16 mouse melanoma model. However, current in-depth knowledge about the complex molecular basis of human melanoma has revealed that this well-established and widely used cell line does not reflect the genetic underpinnings of human melanoma. Instead, preclinical animal models used for efficacy testing of novel immunotherapeutic modalities should be founded on relevant human biology. To help achieve this, the mutational landscape of human melanoma should be modeled on mouse hosts with intact immune systems (e.g., C57BL/6 mice), allowing the evaluation of the effects of the immune system and host-tumor interactions. Ideally, a tumor exhibiting intrinsic immunogenicity on an immunocompetent host would result in the intratumoral recruitment of cellular elements comprising both the innate and adaptive arms of the host immune system. This desired attribute could recapitulate the failure of host immunologic barriers to impede effective antitumor responses in humans.

In addition, the presence of tumor infiltrating lymphocytes in immunocompetent mouse models would recapitulate the occurrence of functionally tolerant T-cell repertoires against unknown tumor antigens in patients with melanoma. A point of concern is the insertion of artificial genetic information (e.g. "non-self" proteins) into tumors that may inadvertently increase their immunogenicity. The artificial aspects of this sort of immunity may not

Pigment Cell Melanoma Res. Author manuscript; available in PMC 2014 July 24.

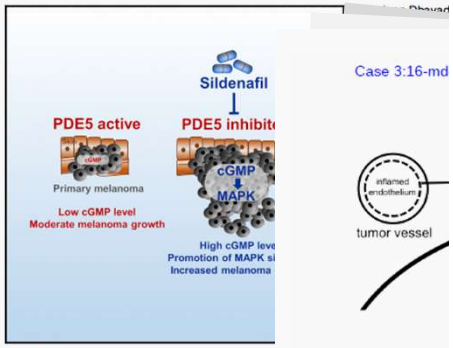
Dhayade *In Vitro*: Does Not Study Sildenafil Alone

Article

Cell Reports

Sildenafil Potentiates a cGMP-Dependent Pathway to Promote Melanoma Growth

Graphical Abstract



Authors

Highlights

- Melanoma cells express a cGMP signaling pathv PDE5
- The cGMP pathway promotes MAPK signaling melanoma cell growth and migration
- PDE5 degrades cGMP and thus acts as a brake growth-promoting cGMP pathway
- The PDE5 blocker sildenafil releases the PDE5 b to increased tumor growth

Dhayade et al., 2016, Cell Reports 14, 2599–26
March 22, 2016 ©2016 The Authors
http://dx.doi.org/10.1016/j.celrep.2016.02.028

Figure S5 (Related to Figure 7). Model of cGMP Signaling in Melanoma Pathogenesis

The scheme illustrates how a bidirectional crosstalk of cGMP and MAPK signaling promotes the switch of non-metastatic cells in primary melanomas to invasive/metastatic cells. CNP is released from endothelial cells (dashed circle) of the inflamed tumor vasculature (upper left) and binds to its receptor, GC-B, on melanoma cells. Thereby, CNP triggers an increase of the intracellular cGMP concentration and activation of cGKIα in the melanoma cells. Via phosphorylation of yet unknown substrate proteins, cGKIα promotes MAPK signaling upstream of MEK (indicated by the bracket), resulting in cells with increased potential for growth, migration, and invasiveness to develop. Degradation of cGMP via PDE5 acts as a "break" in this switching process. However, a persistent increase in MAPK signaling, for instance, by sustained activity of the CNP-cGMP-cGKI cascade and/or by somatic gain-of-function mutation of BRAF to V600E-BRAF (Arozarena et al., 2011), results in downregulation of PDE5 at the transcriptional level. This releases the PDE5 "break", thus, establishing a feed-forward, self-reinforcing loop that further enhances the aggressiveness of the melanoma cells. The PDE5 "break" can also be released pharmacologically by the PDE5 inhibitor sildenafil. Note that CNP and sildenafil might act mainly on cells of the primary tumor to increase their metastatic potential, and that "switched" metastatic cells might lose expression of both cGKI and PDE5. Note also that the present study has analyzed the effects of exogenously supplied CNP. The role of inflamed tumor vessels as the source of endogenous CNP in melanomas needs to be established in future studies. U0126 is a MEK inhibitor used in the present study. RTK stands for receptor tyrosine kinase.

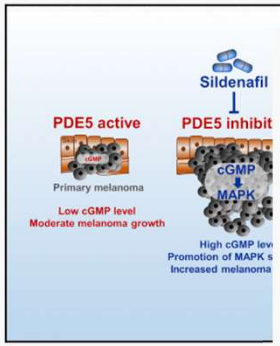
The role of inflamed tumor vessels as the **source of endogenous CNP** in melanomas needs to be **established in future studies**.

Dhayade *In Vitro*: Does Not Study Sildenafil Alone

Cell Reports

Sildenafil Potentiates a cGMP-Depe
to Promote Melanoma Growth

Graphical Abstract



Highlights

- Melanoma cells express a cGMP signaling path PDE5
- The cGMP pathway promotes MAPK signaling melanoma cell growth and migration
- PDE5 degrades cGMP and thus acts as a brak growth-promoting cGMP pathway
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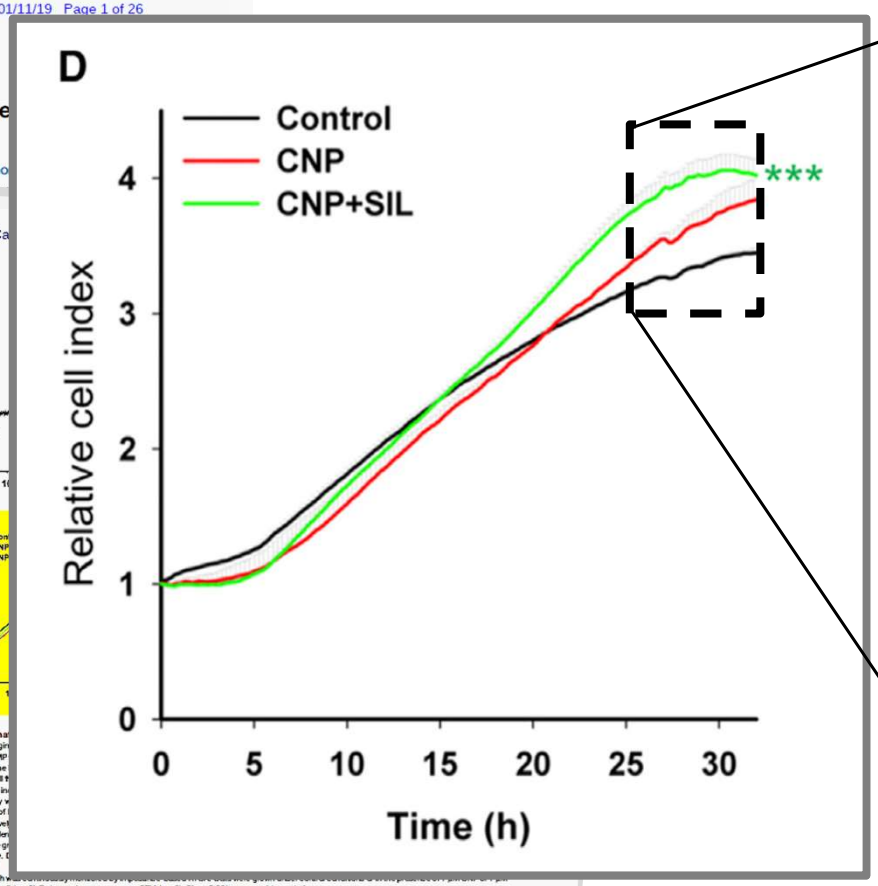
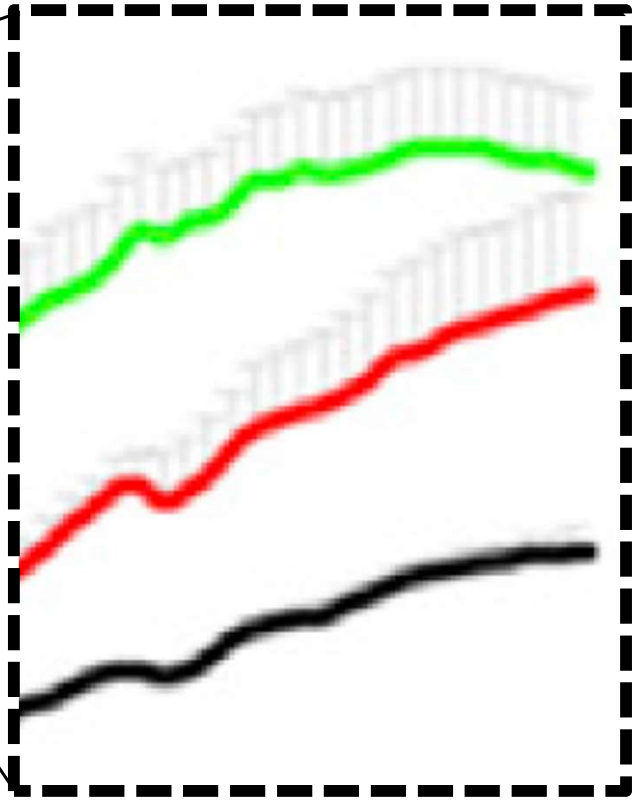
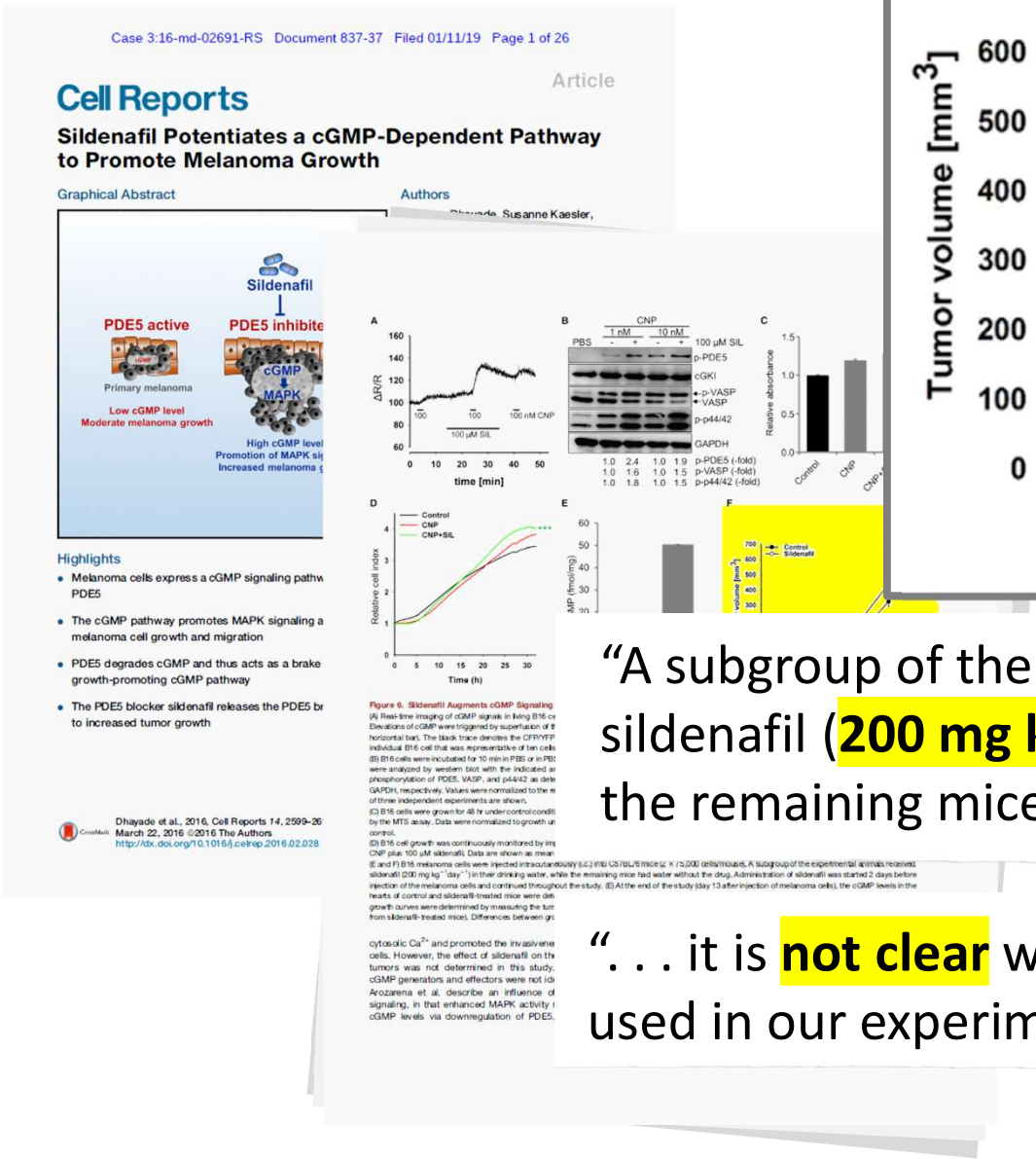
Dhayade et al., 2016, Cell Reports 14, 2599–2
March 22, 2016 ©2016 The Authors
http://dx.doi.org/10.1016/j.celrep.2016.02.028

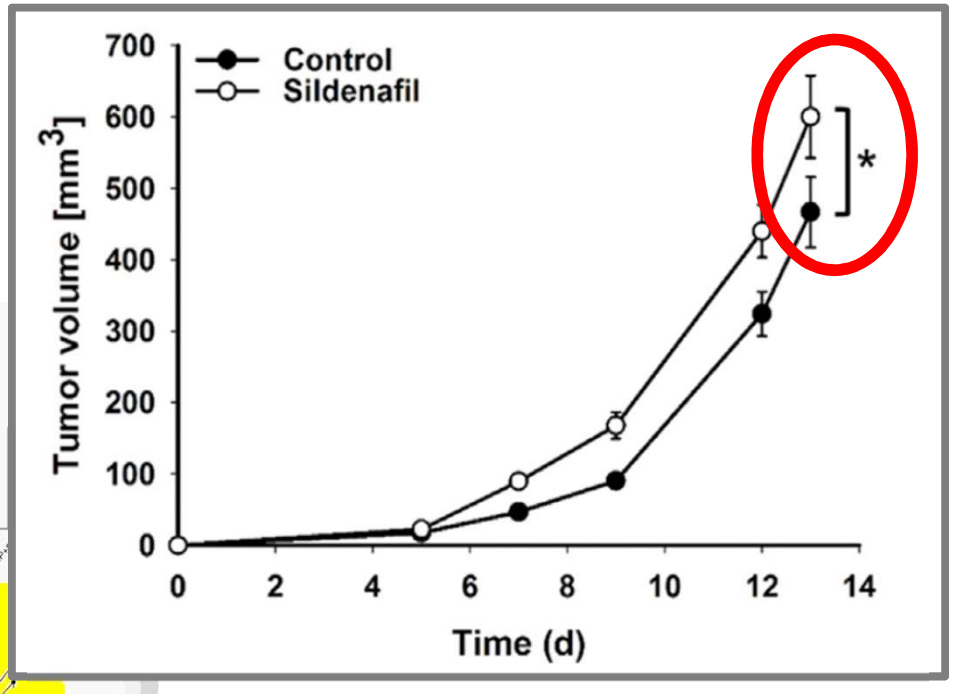




cytosolic Ca^{2+} and promoted the invasiveness of the melanoma cells. However, the effect of sildenafil on the growth of primary tumors was not determined in this study, and the potential cGMP generators and effectors were not identified. Thus, while Arozarena et al. describe an influence of MAPK on cGMP signaling, in that enhanced MAPK activity results in increased cGMP levels via downregulation of PDE5, our study reports vice versa that cGMP also impacts MAPK signaling, in that increased levels of cGMP enhance the activity of MAPK via cGKI. Arozarena and colleagues could not detect cGKI expression in seven BRAF mutant melanoma cell lines analyzed in their study (Arozarena et al., 2011). Our expression analysis of a panel of human melanoma cell lines and tumor sections of human melanoma patients also indicated that cGKI is probably not the only

Supratherapeutic Sildenafil Dosing





"A subgroup of the experimental animals received sildenafil (**200 mg kg/day**) in their drinking water, while the remaining mice had water without the drug."

". . . it is **not clear** whether the sildenafil concentration used in our experiments is also reached **in patients**. . ."

Direct Effects: PDE-5 Inhibitors and Melanoma

ARTICLE
Journal of Cellular Biochemistry 113:2738–2743 (2012)

PDE5 Inhibitor Promotes Melanin Synthesis Through PKG Pathway in B16 Melanoma Cells

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ABSTRACT

PDE inhibitors could increase cellular cGMP levels and are used to treat erectile dysfunction as well as pulmonary arterial hy production was reported to be necessary for UVB-induced melanin synthesis, however, the effect of PDE5 inhibitor on mela not been examined. We found that PDE5 inhibitor (sildenafil or vardenafil) and the cGMP analog 8-CPT-cGMP phosphorylation, leading to increased tyrosinase expression and melanin synthesis, which was counteracted by KT cGMP-dependent protein kinase (PKG) inhibitor. However, KT5823 did not affect cAMP-elevating agent-mediated m indicating that KT5823 selectively inhibited cGMP-induced melanin synthesis. This is the first study to find that PDE5 inhib melanin synthesis and reveal that PKG-dependent CREB phosphorylation and tyrosinase expression is involved in cGMP- synthesis. Our results suggest that PDE5 inhibitor may be beneficial for the treatment of hypopigmentation diseases. J. Cel 2738–2743, 2012. © 2012 Wiley Periodicals, Inc.

KEY WORDS: MELANIN SYNTHESIS; PDE5 INHIBITOR; PKG; TYROSINASE

Melanin is an important skin pigment in human and contributes significantly to the health of an individual [Gilchrest, 1989; Sturm, 2002]. Lack or decreased levels of melanin in human lead to many skin diseases, termed hypopigmentary disorders including vitiligo and gray hair [Hartmann et al., 2004; Dessinioti et al., 2009]. There are several treatments for hypopigmentation diseases, but there remain some problems, such as poor efficacy and severe side effects [Hartmann et al., 2004; Hercogova et al., 2007]. Thus the search for new types of treatments of hypopigmentation diseases with high efficacy and low toxicity is warranted.

Melanin is synthesized in melanocytes via a cascade of enzymatic reactions controlled by tyrosinase [Hearing, 1999; Kim et al., 2010]. Tyrosinase is the rate-limiting enzyme of melanin synthesis that catalyses the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to dopaquinone [Slominski et al., 2004]. Melanin synthesis is stim variety of intrinsic and extrinsic factors, including agents, UVB, and a wide variety of growth facto [Friedmann and Gilchrest, 1987; Yasumoto et al., 2009; Ho et al., 2010]. cAMP pathway plays a regulation of melanogenesis through activation of protein kinase (PKA) and cAMP response elemen transcription factor, which induced an up-regulat expression and the stimulation of melanogenes et al., 2004; Park et al., 2009].

For the first time in 1996, the second messenge to be required for melanin synthesis induced by increase cGMP content in melanocytes [Rome 1996]. Phosphodiesterase 5 (PDE5) is the pre responsible for cGMP hydrolysis in various type [Kass et al., 2007]. PDE5 inhibitors such as sildenafil

Xiaodong Zhang and Guirui Yan contributed equally to this work.
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Journal of Cellular Biochemistry

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3′,5′-Cyclic Nucleotide Phosphodiesterase in Tumor Cells as Potential Target for Tumor Growth Inhibition

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ABSTRACT

Isoenzymes of 3′,5′-cyclic nucleotide phosphodiesterase (PDE) have been characterized in B16 murine melanoma cells and MCF-7 human mammary carcinoma cells. Separation of soluble phosphodiesterase activity by fast protein liquid chromatography on a Mono-Q column resolved three isoenzymes, MCF-7 cells contained a cyclic GMP-specific isoenzyme (PDE-V), a cyclic GMP-activable isoenzyme (PDE-II), and a cyclic AMP-specific isoenzyme (PDE-IV). B16 cells contained a cyclic GMP-specific isoenzyme (PDE-V), a Ca^{2+}/calmodulin-activated isoenzyme (PDE-I), and a cyclic AMP-specific isoenzyme (PDE-IV).

A series of PDE inhibitors was tested for their activity spectrum on PDE isoenzymes. Inhibition of PDE activity in B16 cells by the new compound DC-TA-46, was found to result specifically from PDE-IV inhibition [50% inhibition (IC_{50}) = 0.03 μM]. Much lower inhibitory activity was observed for DC-TA-46 toward PDE-I (IC_{50} = 5 μM) and PDE-V (IC_{50} = 14 μM).

DC-TA-46 was found to inhibit growth of B16 melanoma and MCF-7 mammary carcinoma cells dose dependently (B16: IC_{50} = 1.7 μM, MCF-7: IC_{50} = 2 μM). At 2 μM concentration, growth inhibition of B16 melanoma cells was 60%, concomitant with a decrease in PDE activity of 63% and an increase in cAMP level of 59%. In contrast, incubation with inhibitors specific for PDE-I and PDE-V resulted only in marginal or undetectable growth inhibition. The results suggest a correlation between PDE-IV inhibition and growth inhibition. PDE-IV thus appears to be a potential new target for antiproliferative treatment.

INTRODUCTION

Multiple forms of PDE[1] have been demonstrated in various tissues or cells and have been characterized on the basis of substrate specificity, sensitivity to calmodulin or phosphodiesterase inhibitors, and kinetic parameters (1–3). However, no data are available yet concerning isoenzyme distribution in MCF-7 and B16 tumor cells. We found three isoenzymes: in MCF-7 cells, PDE-II, PDE-IV, and PDE-V; in B16 cells, PDE-I, PDE-IV, and PDE-V. Isoenzyme nomenclature follows that introduced by Beavo (2).

Each form of isoenzyme has a unique role in the regulation of the intracellular level of cyclic nucleotides. cAMP is a positive intracellular signal for cell proliferation in many differentiated cells (4, 5). In many tumor cells, however, cAMP is a negative messenger for proliferation, showing a much lower basal level than in normal cells (5). Some data indicate that the activity of 3′,5′-cyclic nucleotide phosphodiesterase is elevated in tumor cells (3, 6). Various agents elevating cAMP have previously been found to inhibit tumor cell growth *in vitro*. PDE inhibitors, especially those of the methylxanthine type, display, however, growth inhibition only at rather high concentrations (5, 7–14).

A new PDE inhibitor, DC-TA-46 (15), with potent inhibitory activity toward PDE from B16 melanoma and MCF-7 mammary carcinoma, was tested for its ... were identified to find ... be relevant for growth ...

MATERIALS AND ...

Preparation of Cell Ex... dures were performed at 0°C buffer, was suspended in ... benzamidine, 0.1 mM phenyl... 0.1 mM *N*-α-*p*-tosyl-L-lysin... peptin, 0.25 M sucrose, 0.05 ... at 1,000 × *g* for 10 min. T... 100,000 × *g* for 60 min to ...

PDE Assay. PDE activit... (16) with slight modificatio... in buffer containing 50 mM ... Incubation was carried out ... [^{3}H]-5′-AMP was precipita... the supernatant was subject... without PDE.

Mono-Q Ion Exchange ... cell extract was loaded at ... (5 × 0.5 cm), preequilibrate... 0.1 mM phenylmethylsulfon... *p*-tosyl-L-lysine chlorometh... Tris/HCl, pH 7.4). After w... eluted at 1 ml/min, using a ... Fractions of 1 ml were coll...

Effects of Ca^{2+}/Calmod... determined in the presence ... cGMP.

Determination of IC_{50} ... sulfoxide at a stock concent... B to provide a range of ... concentration in the assay ... concentration from the do... were performed in duplica...

Kinetic Parameters. ... eluting from the Mono-Q ... final concentration of 10 n... 0.05 to 100 or 500 μM. A... that no more than 15% of ... from Hanes and Linewe...

Cell Culture. Cells ... CO_2). B16 cells were gr... fetal calf serum and per... same medium containi... Cells (3–4 × 10^5/96... medium was replaced ... every 48 h throughou... performed in triplicate ... and was ...

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ANTICANCER RESEARCH *30*: 355-358 (2010)

Expression and Role of Phosphodiesterase 5 in Human Malignant Melanoma Cell Line

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Abstract. *Background: Eleven phosphodiesterase (PDE) gene families (PDE1-11) have been identified, and some PDE isoforms are selectively expressed in various cell types. Previously, we reported PDE1, PDE3 and PDE4 expressions in human malignant melanoma cells. However, the expression and role of PDE5 in malignant melanoma cells is not clear. Therefore, we characterized PDE5 in human malignant melanoma MAA cells. Materials and Methods: PDE5 activity and PDE5A mRNA expression were investigated in MAA cells. The full open reading frames for human PDE5A1 were sequenced. Effects of PDE5 inhibitors on cell growth were determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assays. Results: PDE5 activity and PDE5A1 mRNA expression were detected in MAA cells. The nucleotide sequence of PDE5A1 was identical to that of human PDE5A1, previously published. Two PDE5 inhibitors inhibited the growth of cells. Conclusion: PDE5A1 mRNA is expressed and may play an important role in the growth of human malignant melanoma MAA cells.*

Eleven phosphodiesterase (PDE) gene families (PDE1-11) have been identified, and some PDE isoforms are selectively expressed in various tissues and cell types, but in different amounts, proportions and subcellular locations. All 11 PDE gene families encode proteins that exhibit a common structural organization, with a conserved catalytic domain in C-terminal portions and divergent regulatory modules and domains in ... the hydrolysis of cyclic nucleotides, PDEs regulate the intracellular concentrations and effects of these secondary messengers. Some PDE families are relatively specific for cAMP (PDEs 4, 7 and 8) or for cGMP (PDEs 5, 6 and 9); others hydrolyze both (PDEs 1-3, 10 and 11) (1, 2, 4).

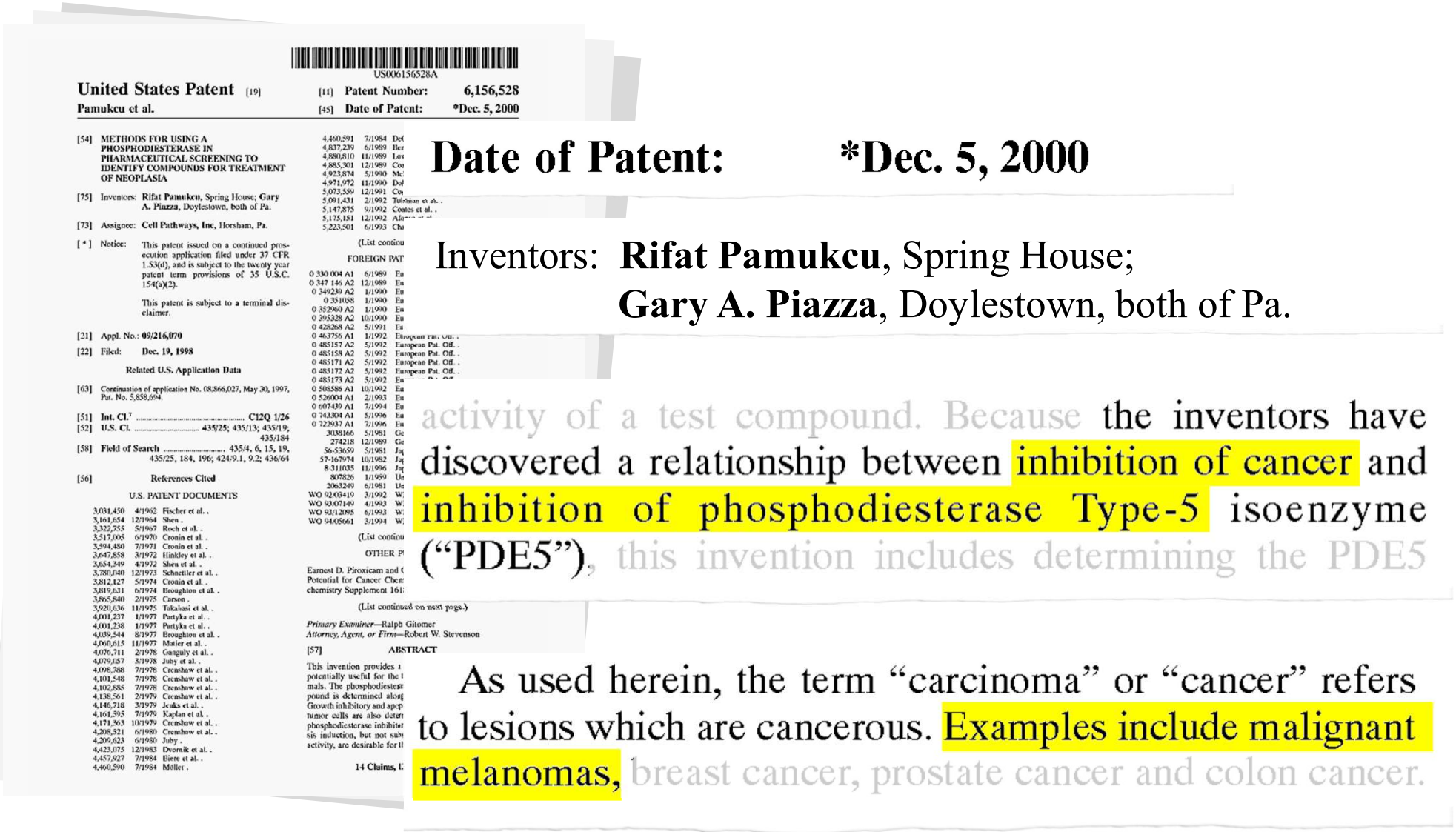
PDE5 is relatively specific for cGMP and is expressed abundantly in vascular smooth muscle, including the pulmonary vasculature and corpus cavernosum of the penis. Three alternatively splicing variants of human *PDE5A* (*PDE5A1*, *PDE5A2* and *PDE5A3*) have been identified and their tissue distribution differs (2, 3, 5). PDE5 inhibitor sildenafil improves penile erection with a minimal risk of side-effects and adverse events in many men with erectile dysfunction (1-3, 5). However, the expression and role of PDE5 in human malignant melanoma cells is not clear. Therefore, we examined *PDE5* in human malignant melanoma MAA cells.

Materials and Methods

Cell culture. Human malignant melanoma MAA cells were established and maintained in RPMI 1640 containing 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA, USA) at 37°C in a humidified 5% CO_2 atmosphere in our laboratory (6).

cGMP PDE activity assay in cell extracts. The cells were seeded at 1×10^6 cells/25-cm^2 flask. After 3 days, the cells were washed twice with phosphate-buffered saline (PBS), harvested with a rubber policeman, and homogenized in ice-cold homogenization buffer (1 ml: 100 mM Tris-HCl, pH 7.6, 150 μg/ml each of pepstatin, leupeptin ...

Dr. Piazza 2000 Patent



US006156528A

United States Patent [19]
Pamukcu et al.

[11] **Patent Number: 6,156,528**
[45] **Date of Patent: *Dec. 5, 2000**

[54] METHODS FOR USING A PHOSPHODIESTERASE IN PHARMACEUTICAL SCREENING TO IDENTIFY COMPOUNDS FOR TREATMENT OF NEOPLASIA

[75] Inventors: **Rifat Pamukcu**, Spring House; **Gary A. Piazza**, Doylestown, both of Pa.

[73] Assignee: **Cell Pathways, Inc**, Horsham, Pa.

[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

This patent is subject to a terminal disclaimer.

[21] Appl. No.: **09/216,070**

[22] Filed: **Dec. 19, 1998**

Related U.S. Application Data

[63] Continuation of application No. 08/866,027, May 30, 1997, Pat. No. 5,858,694.

[51] **Int. Cl.**[7] **C12Q 1/26**
[52] **U.S. Cl.** **435/25**; 435/13; 435/19; 435/184
[58] **Field of Search** 435/4, 6, 15, 19, 435/25, 184, 196; 424/9.1, 9.2; 436/64

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Earnest D. Piroxicam and [illegible] Potential for Cancer Chem[illegible] chemistry Supplement 161:

(List continued on next page.)

Primary Examiner—Ralph Gitomer
Attorney, Agent, or Firm—Robert W. Stevenson

[57] **ABSTRACT**

This invention provides [illegible] potentially useful for the t[illegible] mals. The phosphodiester[illegible] pound is determined along[illegible] Growth inhibitory and apop[illegible] tumor cells are also deter[illegible] phosphodiesterase inhibitor[illegible] sis induction, but not subs[illegible] activity, are desirable for t[illegible]

14 Claims, [illegible]

Date of Patent: *Dec. 5, 2000

Inventors: **Rifat Pamukcu**, Spring House;
Gary A. Piazza, Doylestown, both of Pa.

activity of a test compound. Because the inventors have discovered a relationship between inhibition of cancer and inhibition of phosphodiesterase Type-5 isoenzyme ("PDE5"), this invention includes determining the PDE5

As used herein, the term "carcinoma" or "cancer" refers to lesions which are cancerous. Examples include malignant melanomas, breast cancer, prostate cancer and colon cancer.

Dr. Piazza 2018 Patent

US010039764B2

(12) **United States Patent**
Piazza

(10) **Patent No.: US 10,039,764 B2**
(45) **Date of Patent: Aug. 7, 2018**

(54) TREATMENT AND DIAGNOSIS OF CANCER AND PDE10A MEASU

(71) Applicar

(72) Inventor:

(73) Assignee

(*) Notice:

(21) Appl. No.: **14/904,632**

(22) PCT Filed: **Jul. 11, 2014**

(86) PCT No.:

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(2) Date:

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US 2016

(60) Provisio
12, 2013

(51) Int. Cl.
A61K 31
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A61K 31
A61K 31
A61K 45
C12Q 1/
G01N 3

(52) U.S. Cl.
CPC ....

(2013.01); *A61K 31/517* (2013.01); *A61K 45/06* (2013.01); *C12Q 1/6886* (2013.01); *G01N 33/57496* (2013.01); *C12Q 2600/118* (2013.01); *C12Q 2600/158* (2013.01); *G01N 2333/916* (2013.01); *G01N 2800/50* (2013.01)

(58) **Field of Classification Search**
CPC A61K 31/415; A61K 31/4709; A61K 31/4745; A61K 31/4155; A61K 31/4409
See application file for complete search history.

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Shawn P. Foley

(57) **ABSTRACT**

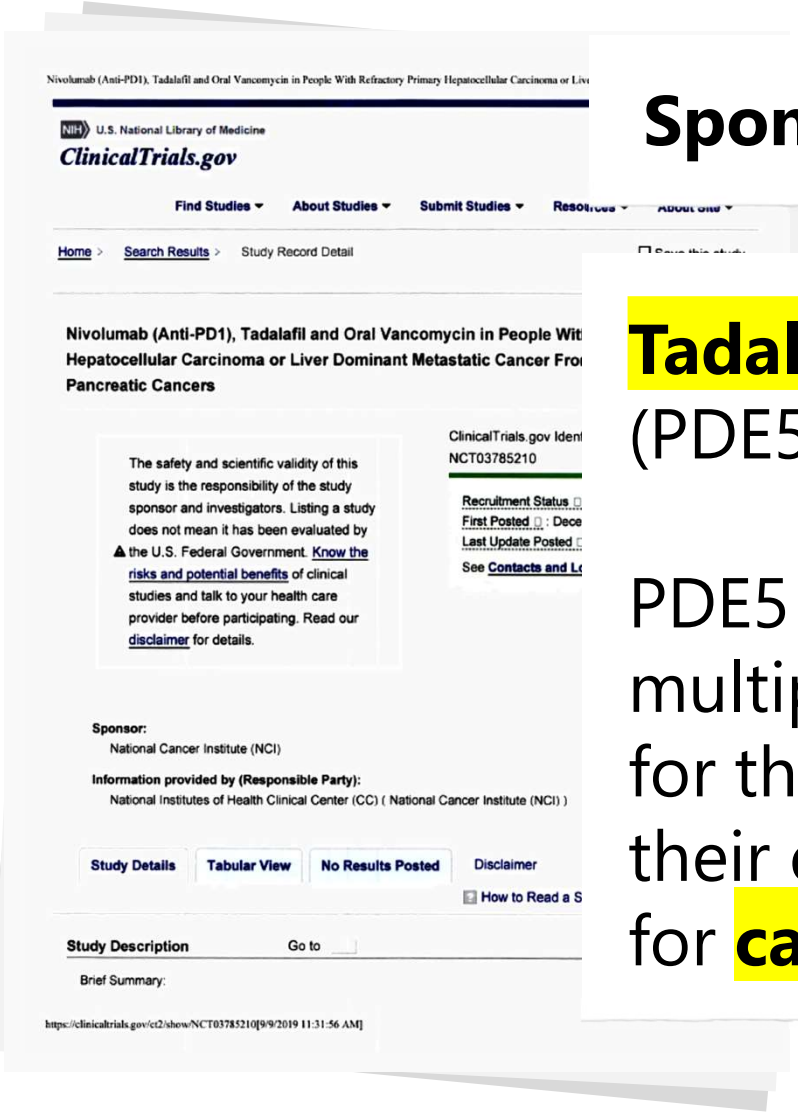
Disclosed are methods for treating cancer and precancerous conditions with PDE10A specific inhibitors and diagnosis of neoplastic diseases based on elevated levels of PDE10A.

8 Claims, 15 Drawing Sheets

Examples of cancers characterized by solid tumors which may be treated include … melanoma

For instance, in some embodiments, the PDE10A inhibitor is co-administered with a PDE5 inhibitor to improve efficacy or reduce the effective dose range of the PDE10A inhibitory. Representative examples of PDE5 inhibitors that may be useful in the practice of the present invention include sildenafil, tadalafil, vardenafil, udenafil, and avanafil or others such as MY5445 or compounds that increase intracellular cGMP levels (e.g., nitric oxide donors or releasing drugs). Examples of yet other PDE5 inhibitors that may be suitable for

Ongoing Research


Nivolumab (Anti-PD1), Tadalafil and Oral Vancomycin in People With Refractory Primary Hepatocellular Carcinoma or Liv

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Pancreatic Cancers

The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Know the risks and potential benefits of clinical studies and talk to your health care provider before participating. Read our disclaimer for details.

ClinicalTrials.gov Ident
NCT03785210

Recruitment Status
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Sponsor:
National Cancer Institute (NCI)

Information provided by (Responsible Party):
National Institutes of Health Clinical Center (CC) (National Cancer Institute (NCI))

Study Details | Tabular View | No Results Posted | Disclaimer
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Brief Summary:

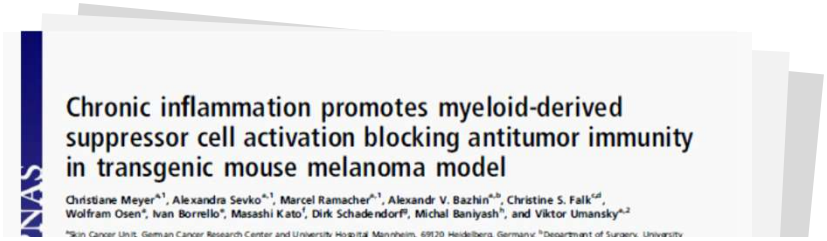
https://clinicaltrials.gov/ct2/show/NCT03785210[9/9/2019 11:31:56 AM]

Sponsor: National Cancer Institute (NCI)

Tadalafil is a phosphodiesterase type 5 (PDE5) inhibitor . . .

PDE5 inhibitors have been examined in multiple malignancies and cancer cell lines for their **direct anticancer activities**, for their efficacy as **chemo-sensitizers** and for **cancer chemoprevention**.

PDE-5 Inhibitors and Immunity: Anti-Melanoma Effects

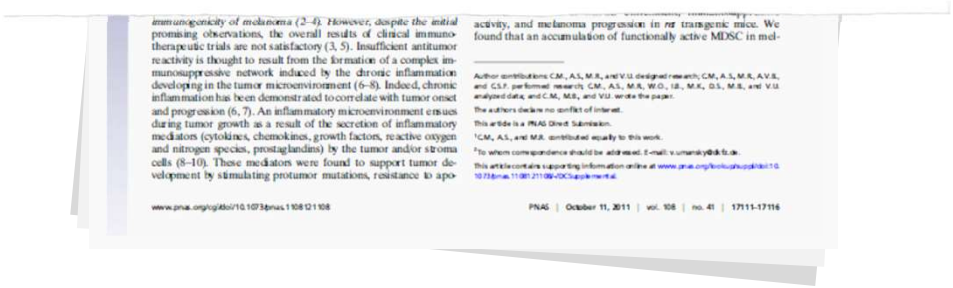


Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model

Christiane Meyer[a,1], Alexandra Sevko[a,1], Marcel Ramacher[a,1], Alexandr V. Bazhin[a,b], Christine S. Falk[c,d], Wolfram Osen[a], Ivan Borrello[e], Masashi Kato[f], Dirk Schadendorf[g], Michal Baniyash[h], and Viktor Umansky[a,2]

"manipulation of the melanoma microenvironment with … **sildenafil**"

"significantly **increased survival** of tumor-bearing mice."



immunogenicity of melanoma (2–4). However, despite the initial promising observations, the overall results of clinical immunotherapeutic trials are not satisfactory (3, 5). Insufficient antitumor reactivity is thought to result from the formation of a complex immunosuppressive network induced by the chronic inflammation developing in the tumor microenvironment (6–8). Indeed, chronic inflammation has been demonstrated to correlate with tumor onset and progression (6, 7). An inflammatory microenvironment ensues during tumor growth as a result of the secretion of inflammatory mediators (cytokines, chemokines, growth factors, reactive oxygen and nitrogen species, prostaglandins) by the tumor and/or stroma cells (8–10). These mediators were found to support tumor development by stimulating protumor mutations, resistance to apoptosis, and melanoma progression in *ret* transgenic mice. We found that an accumulation of functionally active MDSC in mel-

Author contributions: C.M., A.S., M.R., and V.U. designed research; C.M., A.S., M.R., A.V.B., and C.S.F. performed research; C.M., A.S., M.R., W.O., I.B., M.K., D.S., M.B., and V.U. analyzed data; and C.M., M.B., and V.U. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]C.M., A.S., and M.R. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: v.umansky@dkfz.de.

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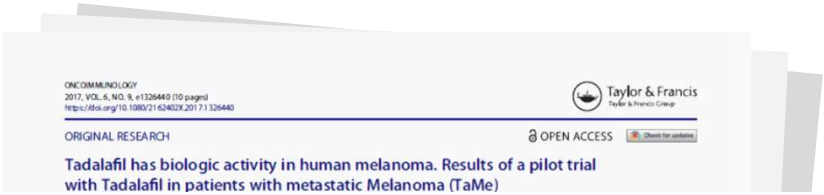

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https://doi.org/10.1080/2162402X.2017.1326440

Taylor & Francis

ORIGINAL RESEARCH

OPEN ACCESS

Tadalafil has biologic activity in human melanoma. Results of a pilot trial with Tadalafil in patients with metastatic Melanoma (TaMe)

"**Tadalafil** led to a stabilization of the disease in 3 of 12 pretreated patients in the palliative setting."

"Our study suggests that the PDE-5 inhibitor **tadalafil can improve clinical outcome of advanced melanoma patients**. . ."

CONTACT Jessica C. Hassel, MD jessica.hassel@med.uni-heidelberg.de Department of Dermatology and National Center for Tumor Diseases (NCT), University Hospital Heidelberg, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany.

Supplemental data for this article can be accessed on the publisher's website.

*These authors contributed equally.